Release of Piperonyl Butoxide and Permethrin from Synergized Ear Tags on Cattle and Effects on Horn Fly Mortality

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ABSTRACT A study was conducted to determine the release rates of piperonyl butoxide (PBO) and permethrin from synergized insecticidal cattle ear tags and their effects on mortality of the horn fly, *Hematobia irritans irritans* (L.) (Diptera: Muscidae). PBO was released from the ear tags at a higher rate than permethrin in both winter and summer trials. The cumulative release of PBO and permethrin from the ear tags at the end of 18 wk in the winter trial was 50.4 and 30.3%, respectively. The cumulative release of PBO and permethrin from the ear tags at the end of 18 wk in the summer trial was 66.7 and 44.7%, respectively. There was a significant correlation between the cumulative daily high ambient temperature (°C) and the cumulative release of both PBO and permethrin. Compared with the susceptible horn fly strain, the permethrin-resistant strain demonstrated 7.9- and 12.8-fold resistance to permethrin at the levels of LC_{50} and LC_{90}, respectively. When exposed to filter paper wipes taken from the shoulders of cattle treated with the PBO-synergized permethrin tags from the summer trial, the resistant strain demonstrated reduced mortality compared with the susceptible strain. The mortality of the resistant strain at 2- and 3-h exposure exhibited a pattern of declining fly mortalities as a result of the decreased release of PBO and permethrin, as well as the decline in the ratio of PBO:permethrin released from the tags after 8 wk. A similar decline in horn fly mortalities was observed in the susceptible strain at 30-min exposure time that coincided with the pattern of reduced release of PBO and permethrin from the ear tags over the course of summer trial.

KEY WORDS insecticidal ear tags, release, permethrin, piperonyl butoxide, resistance

Introduction of the cattle insecticidal ear tag in the late 1970s provided a simple, practical, inexpensive, long-lasting control of livestock ectoparasites. Numerous studies have shown that the insecticidal tags containing a variety of pesticides provided excellent control of the horn fly, *Hematobia irritans irritans* (L.) (Diptera: Muscidae), and significant reductions of the face fly, *Musca autumnalis* De Geer, on cattle (Ahrens 1977, Ahrens and Cooke 1979, Beadles et al. 1979, Schmidt and Kunz 1980, Gladney 1981, Williams et al. 1981, Miller et al. 1984). These tags also provided control of the Gulf Coast tick, *Amblyomma maculatum* Koch, and the southern cattle tick, *Boophilus microplus* Canestrini (Gladney 1976, Ahrens et al. 1977, Ahrens and Cooke 1978, Davey et al. 1980).

Horn fly resistance to the insecticides being used in the ear tags began to occur after 3 yr of widespread use (Sheppard 1983, 1984; Quisenberry et al. 1984; Schmidt et al. 1985). Shortly thereafter, resistance was confirmed in horn flies across the United States The unprecedented rate of development of resistance and the levels of resistance reported, particularly to pyrethroids, were attributed to insecticidal ear tag use (Kunz and Kemp 1994). The slow release and continuous delivery of the insecticide from the ear tags exposed multiple generations of horn flies to heavy selection pressures. Moreover, the rapid acceptance and widespread use of insecticide tags meant that greater proportions of the horn fly population were being pressured than with any previous technology; thus, reducing susceptible refugia. Strategies to counter the further development of resistance included 1) eliminating or reducing the use of the insecticidal tags, 2) using alternative application technologies (spray, dust, pour-ons, backrubbers) only when horn fly populations exceed the suggested economic threshold, and 3) alternating chemistries in the insecticidal ear tags to those with different modes of action. A recent ear tag strategy involves the use of a synergist to enhance the efficacy of the pesticide in the tags and prolong the utility of the insecticidal tag technology.

Insecticidal ear tags are typically formulated by incorporating the pesticide with or without a plasticizer into a polymer matrix (most commonly polyvinyl

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chloride). The pesticide is released over an extended period through a diffusion process and deposited onto the hair coat of the cattle. Two key parameters critical to the effectiveness of tags are the rate of release of the pesticide and the change in rate of release over time on the animal. Miller et al. (1983) described the pesticide release patterns of selected pyrethroids and related these data to efficacy against the horn fly. They demonstrated that the release of the pesticide from the homogenous monolithic ear tag systems followed Fick's laws of diffusion and could be described by mathematical expressions derived by Baker and Lonsdale (1974). The equations involved both the diffusion coefficient of the permeator and the thickness of the tag. The diffusion coefficient is a characteristic of the specific pesticide (permeator). Therefore, the formulation of an insecticidal ear tag with both a synergist and a pesticide is likely to result in the each component diffusing from the tag at a different rate. Tags formulated to initially deliver the optimal ratio of synergist:pesticide could over time result in a less than optimal and ineffective ratio over time. The objective of this study was to describe the pattern of release of piperonyl butoxide (PBO) and permethrin from ear tags and its effects on horn fly mortality.

Methods and Materials

This study was conducted at the USDA, Agricultural Research Service, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX, as part of a research program to develop more effective methods of controlling ectoparasites on cattle. Two separate tests, one test during the winter (November 2001–March 2002) and one test during the summer (May–September 2002), were conducted using the same experimental protocol. In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Institutional Animal Use and Care Committee of the Knipling-Bushland U.S. Livestock Insects Research Laboratory.

Animals and Insecticidal Ear Tags. Five Hereford heifers weighing ≈200 kg were treated with the Atroban Extra insecticide ear tags (Schering-Plough Animal Health Corp., Union, NJ). Each heifer was treated with a tag placed on the backside of each ear so as to maximize contact with the hair coat of the animal. These tags contained a nominal 10% permethrin and 13% PBO in a polyvinyl chloride matrix and weighed ≈10 g each. Just before installing the tags on each animal, the tags were numbered 1–5 and identified as right or left signifying the heifer and the placement of the tag on each animal. The tagged animals were maintained in a fenced area where they could move freely.

Sample Collection and Analysis. Each tag was sampled with a hole punch that removed a 6-mm diameter sample disc weighing ≈27 mg. The samples were individually wrapped in aluminum foil and labeled for subsequent analyses of permethrin and PBO concentration. To determine concentration of each component in ear tag samples, the discs were weighed and placed in 10 ml of acetonitrile (Burdick & Jackson, Muskegon, MI) in a capped glass vial. The samples were allowed to stand for a minimum 24 h with occasional agitation to allow the concentration of PBO and permethrin in the plastic disc to reach equilibrium. The resultant acetonitrile extract was diluted appropriately, filtered through a 0.45-μm filter, and subjected to high-performance liquid chromatography (HPLC) analysis. A 20-μl aliquot of the extract was injected into an Agilent HPLC 1100 series system (Agilent Technologies, Waldbronn, Germany) equipped with a UV-visible variable wavelength detector set at 237 nm. The analytical column was a Waters Nova-Pak C18, 4-μm, 5- by 100-mm Radial-Pak Cartridge. The mobile phase was HPLC grade acetonitrile:water (85:15) at a flow rate of 1.0 ml/min.

The Agilent system computed and compared the area under the PBO and permethrin peaks of the samples with that of standards and calculated the concentration of each remaining in the extracted sample, and then calculated for that remaining in the ear tag. From these data we calculated the cumulative release of PBO and permethrin and the released PBO to permethrin ratio at each sample time. The tags were sampled weekly through the first 4 wk posttreatment and biweekly thereafter through the 18 wk posttreatment.

Bioassays. Susceptibility of the susceptible reference strain (Kerrville) and the permethrin-resistant strain (Oklahoma) of the horn fly were determined using a standard petri dish bioassay technique (Li et al. 2003). During the summer trial when horn flies would normally be present, filter paper wipes were taken from each shoulder of each animal each time the tags were sampled. A 9-cm-diameter piece of filter paper was rubbed 10 times over an approximate 250-cm sq area on each shoulder. In total, 10 filter papers were generated at each sampling date. The filter papers were wrapped in aluminum foil, labeled, and stored at −20°C for bioassays at the end of the season. Bioassays were conducted using a susceptible reference strain (Kerrville) and a permethrin-resistant strain (Oklahoma) at room temperature. Five filer papers were used for testing the susceptible flies, and the other five filter papers for testing resistant flies. Twenty-five 3–4-d-old horn flies either from the susceptible strain or the resistant strain were exposed to the treated filter paper in each of the petri dishes. Mortality of flies in each petri dish was recorded at 30 min, 1 h, 2 h, and 3 h after introduction of flies.

Data Analysis. Data on cumulative release of PBO and permethrin and the ratio of PBO:permethrin were subjected to regression analyses with time as the independent variable using JMP software (SAS Institute 2000). The same software was also used in regression analysis of the cumulative release of PBO and permethrin with cumulative daily high temperature (°C) as the independent variable. Dose–mortality responses of both the susceptible and resistant horn fly strains were analyzed with the POLO-PC program (LeOra Software 1987). Resistance ratio (RR) was

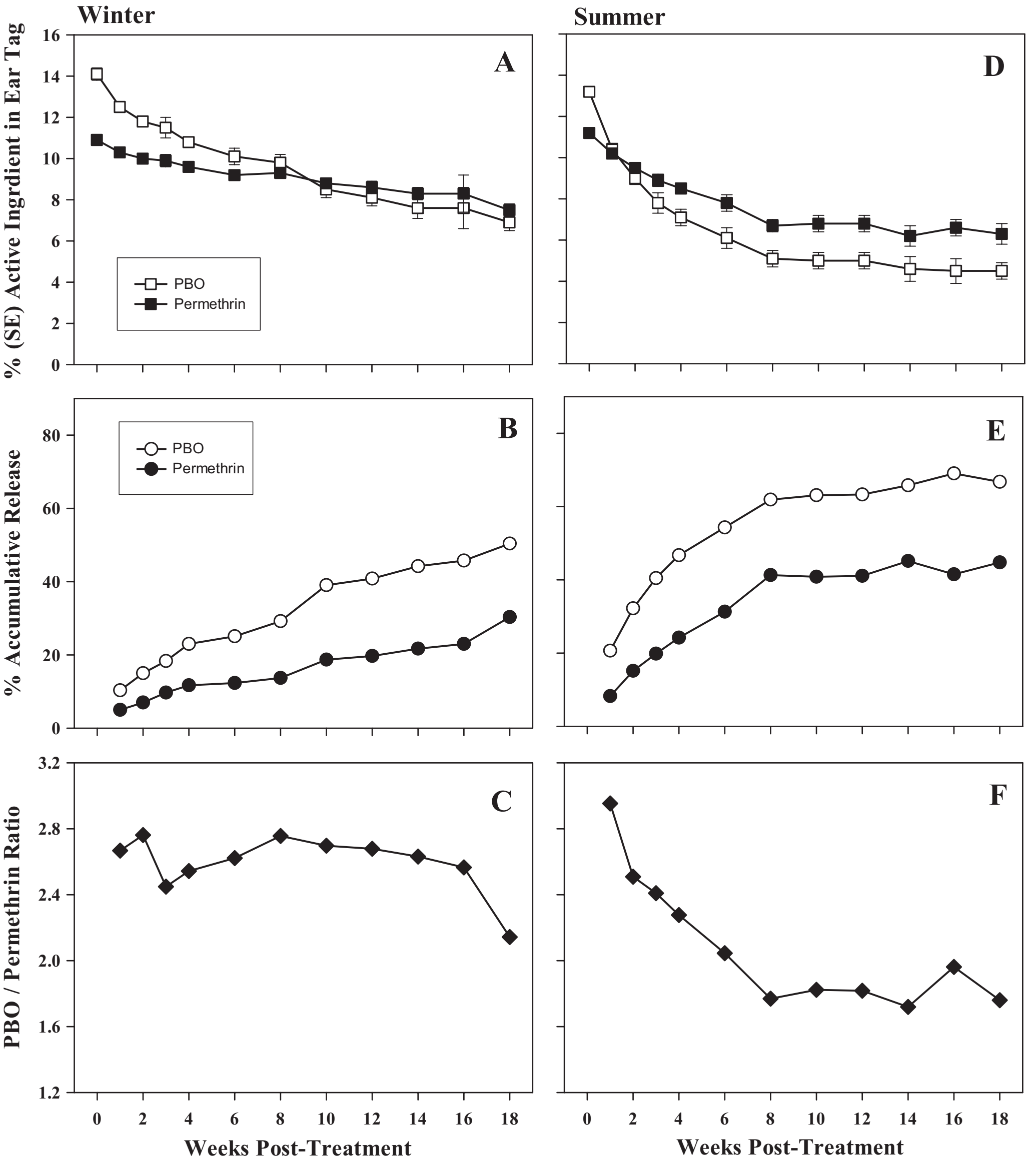

Fig. 1. Changes in mean percentage of PBO and permethrin in the ear tags (n = 10; A and D), accumulative percentage of release of PBO and permethrin from the ear tags (B and E), and the ratios of accumulative percentage of PBO and permethrin released from the ear tags (C and F) during 18 wk of study in the winter (A–C) and summer (D–F) seasons.

calculated by dividing the LC_{50} or LC_{90} value of the resistant horn fly strain with the corresponding LC_{50} or LC_{90} value of the susceptible strain. Fly mortalities from exposure to filter papers treated by the shoulder rubs at various times after tagging were compared using the JMP software (SAS Institute 2000).

Results

Release of PBO and Permethrin from Ear Tags. In the winter trial, the concentration of synergist and insecticide in the Atroban Extra ear tags declined from an initial 14.1 ± 0.3% PBO and 10.9 ± 0.2% permethrin in the new tags to 6.9 ± 0.4% PBO and 7.5 ± 0.3% permethrin after 18 wk on the cattle (Fig. 1A). A linear relationship between cumulative release (percentage) and time (weeks) was determined for both PBO (Y = 11.44 + 2.29X; F = 295.9; df = 1, 9; $P < 0.001$; $r^2 = 0.97$) and permethrin (Y = 4.79 + 1.28X; F = 239.9; df = 1, 9; $P < 0.001$; $r^2 = 0.96$) (Fig. 1B). At the end of the season, 50.4% PBO and 30.3% of permethrin were depleted from the ear tags. The ratio of PBO to permethrin cumulatively released at each sampling remained relatively stable, ranging from 2.14 to 2.76 (Fig. 1C).

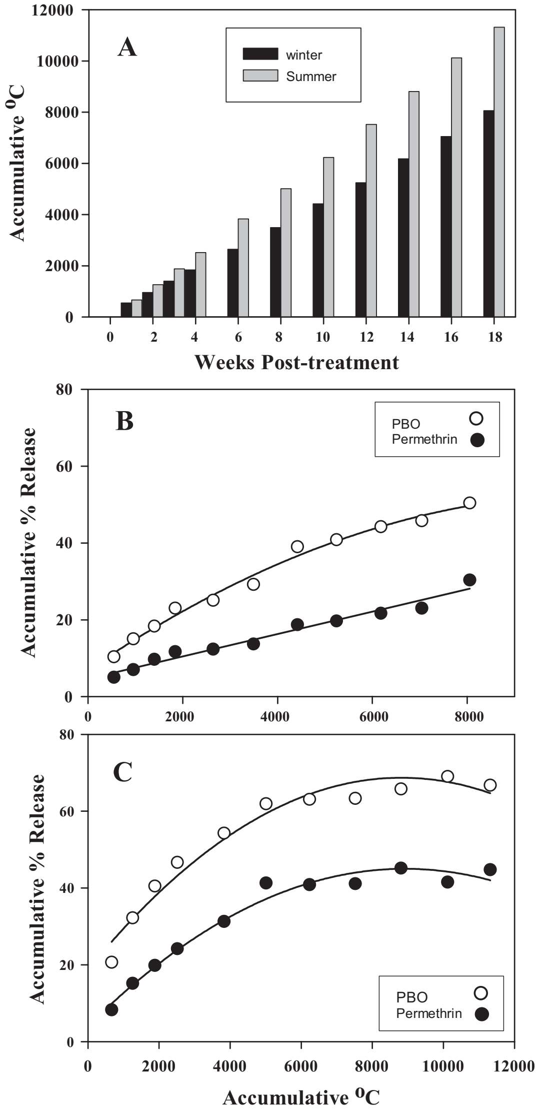

Fig. 2. Accumulative daily high temperature (A) and effects of accumulative daily high temperature on the accumulative percentage of release of the active ingredients (PBO and permethrin) during the winter (B) and summer (C) trials.

In the summer trial, the concentration of synergist and insecticide in the Atroban Extra ear tags declined from an initial 13.2 ± 0.1% PBO and 11.2 ± 0.1% permethrin in the new tags to 4.5 ± 0.4% PBO and 6.3 ± 0.5% permethrin after 18 wk on the cattle (Fig. 1D). A linear relationship between cumulative release (percentage) and time (weeks) was observed during the first 8 wk for both PBO (Y = 20.42 + 5.57X; F = 64.5; df = 1, 4; P < 0.005; r^2 = 0.94) and permethrin (Y = 5.34 + 4.49X; F = 464.8; df = 1, 4; P < 0.001; r^2 = 0.99) (Fig. 1E). Little increase in cumulative release of either PBO or permethrin was observed after week 8. At the end of the season, 66.7% PBO and 44.7% of permethrin were depleted from the ear tags. The ratio of PBO to permethrin cumulatively released at each sampling decreased from an initial value of 2.95–1.77 at 8 wk posttagging, in a linear manner (Y = 2.93 − 0.15X; F = 56.8; df = 1, 4; P < 0.005; r^2 = 0.94), and it remained relatively stable (1.72–1.96) afterward (Fig. 1F).

Effects of Ambient Temperature. The daily average high ambient temperature at the experiment site during the winter and summer trials was 63.4 ± 10.2 and 89.1 ± 5.7°C (n = 127), respectively. The cumulative high temperatures at each of the sampling dates for both trials are shown in Fig. 2A. Regression analysis of winter trial data revealed a binomial relationship between cumulative percentage of release of PBO and cumulative degrees Celsius (F = 352.7; df = 2, 8; P < 0.001; r^2 = 0.99), whereas a linear relationship was found between accumulative percentage of release of permethrin and accumulative degrees Celsius (F = 274.3; df = 1, 9; P < 0.001; r^2 = 0.97) (Fig. 2B). In the summer trial, a binomial relationship was found between the accumulative percentage of release and accumulative degrees Celsius for both PBO (F = 108.1; df = 2, 8; P < 0.001; r^2 = 0.96) and permethrin (F = 153.7; df = 2, 8; P < 0.001; r^2 = 0.97 (Fig. 2C).

Levels of Permethrin Resistance. Compared with the LC_{50} and LC_{90} values of the susceptible horn fly strain, the resistant horn fly strain demonstrated 7.9- and 12.8-fold resistance, respectively (Table 1).

Change of Horn Fly Mortality Associated with Decreased PBO:Permethrin Ratio. The mortalities of horn flies resulting from the bioassay of the filter paper wipes taken during the summer trial are shown in Fig. 3. Although the filter paper bioassays continued to show nearly 100% mortality of the Kerrville susceptible strain at 2-h exposure throughout the 18-wk trial, the 30-min exposure bioassay demonstrated the declining mortality in a pattern similar to the decline in the pattern of release of PBO and permethrin from the tags. There was a significant linear correlation between the mortality of the susceptible strain at 30 min and the ratio of cumulative PBO and permethrin released from ear tags (Y = −95.0 + 70.31X; F = 22.5; df = 1, 9; P < 0.001; r^2 = 0.71). Likewise, there was a significant linear correlation between the mortality of

Table 1. Results of permethrin bioassays with the susceptible and the resistant horn fly strains

Horn fly strain	n	Slope (SE)	χ^2 (df)	LC_{50} (95% CI)[a]	RR	LC_{90} (95% CI)[a]	RR
Susceptible	525	6.50 (0.52)	23.72 (16)	1.45 (1.35–1.55)	1	2.28 (2.07–2.60)	1
Resistant	825	3.16 (0.25)	90.52 (28)	11.43 (9.53–14.74)	7.9	29.10 (20.79–52.30)	12.8

[a] CI, confidence interval.

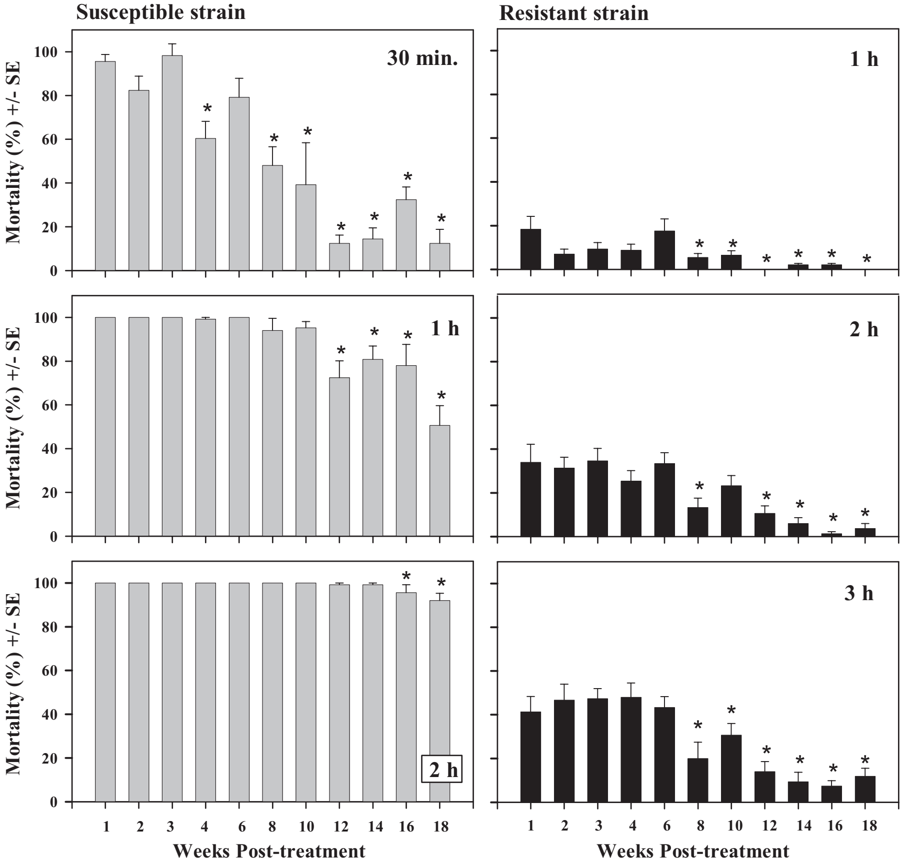

Fig. 3. Mean mortalities of horn flies exposed to filter papers wiped on cattle at different times after the ear tags were applied during the summer. The notation in each figure was the exposure time of the filter paper bioassay. The asterisk (*) indicates significantly different from the mean mortality of the first sample ($P < 0.05$; t-test).

the resistant strain at 2 h ($Y = -31.90 + 24.65X$; $F = 11.5$; df = 1, 9; $P < 0.01$; $r^2 = 0.56$) or 3 h ($Y = -36.32 + 31.23X$; $F = 10.7$; df = 1, 9; $P < 0.01$; $r^2 = 0.54$) and the ratio of cumulative PBO and permethrin released from ear tags.

Discussion

This study demonstrated differences in the release rates of PBO and permethrin from the ear tag, and seasonal effects on those release rates. In the winter season, the cumulative percentage of release of both PBO and permethrin showed a linear increase against time, and the rate of PBO release (slope = 2.29) was nearly twice that of permethrin (slope = 1.28) (Fig. 1B). However, the ratio of cumulatively released PBO and permethrin remained relatively unchanged throughout the season. During first 8 wk of the summer trial, there was a higher rate of PBO and permethrin release (slope = 5.57 and 4.49, respectively) from ear tags, leading to a linear decrease in the PBO: permethrin ratio with time. Only a slight release of PBO and permethrin occurred after week 8, when 61.9% of PBO and 41.3% of permethrin were depleted from the ear tags. The overall higher release rates of PBO and permethrin in the summer season may be partially explained by warmer ambient temperatures, because temperature is known to affect the rate of diffusion and release of insecticides from the polymer matrix (Miller et al. 1983).

The results of regression analysis of cumulative percentage of release of PBO and permethrin against cumulative high daily temperature suggest that the diffusion of both PBO and permethrin was temperature-dependent. The cumulative release rate would be dependent upon the diffusion rate of the solute within the ear tag and the removal of solute from the surface of the tag (Miller et al. 1983). Removal of the solute from the surface of the tag would be behaviorally dependent, and it may be assumed that grooming behavior would increase during summer when pest populations were greater. Because the animals were not individually maintained, the deposition of permethrin and PBO on the shoulder wipes also may have been affected by direct animal to animal contact. Most, if not all, of the PBO and permethrin depleted from the ear tags were presumably deposited on the animal coat/hairs. Results of bioassays using filter paper wipes was an independent measure of the amount of PBO and permethrin released from ear tags and deposited on the animal. We found significant correlations between fly mortality data and the ratio of cumulatively released PBO and permethrin in both the susceptible strain at 30 min and the resistant strain (2 and 3 h), validating the techniques we used in this study. As demonstrated in the summer trial, the ratio of the cumulative release of PBO and permethrin was important to maintain effective permethrin toxicity against resistant flies. We observed a significant decrease in fly mortality when the ratio of PBO and permethrin dropped below 2 in the summer trial. The efficacy of ear tags against horn flies was likely affected by both the PBO:permethrin ratio and the amount of permethrin deposited on the animals. Although the overall release rate was lower for both PBO and permethrin in the winter, the ratio of released PBO and permethrin was in the range of 2.14–2.76 for the entire season. It remains to be determined whether control efficacy of the ear tags during summer could be improved by a formulation that would reduce the rate of PBO release and maintain a higher PBO: permethrin ratio (>2). Raffa and Priester (1985) discussed the potential role of synergists in insecticide resistance management both as a means of delaying the onset of resistance and of combating resistance once it has developed. With the development of pyrethroid resistance by the horn fly, the use of synergists in ear tags has been proposed to be a logical and defensive approach to combating resistance and retaining the insecticidal ear tag as a useful control tool. The incorporation of the synergist with the pesticide in a tag may be the simplest and most practical approach to manufacturing the tags. However, the decline in the ratio of PBO: permethrin demonstrated in this study points to the need for improvement in the way a synergist might be delivered. Ideally, as the pesticide release declines over time, because it invariably will from a homogenous polymeric formulation, it would be desirable to increase the ratio of synergist to pesticide to enhance efficacy of the lower dose of pesticide being delivered. In this study, we demonstrated that the ratio actually declined, in addition to a decrease in the pesticide delivered over time from this monolithic tag. It is therefore conceivable that at some point in the life of the tag, the PBO in the tag is of little or no value in enhancing control.

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